

What is claimed is:

1. An immunogen, characterized in that said immunogen comprises a polypeptide sequence comprising amino acid sequence 1, amino acid sequence 2 and amino acid sequence 3, and these amino acid sequences 1,2 and 3 are covalently linked together by linking peptides consisting of several amino acid residues; said amino acid sequence 1 is the sequence of Th cell epitope; said amino acid sequence 2 is the sequence of a CTL epitope from hepatitis B virus; and said amino acid sequence 3 is the sequence of B cell epitope from hepatitis B virus.
2. An immunogen according to claim 1, characterized in that said amino acid sequence 1 is the amino acid sequence at position 830-843 of the Th cell epitope derived from tetanus toxoid or variant sequences thereof, or the universal Th cell epitope of PADRE; said amino acid sequence 2 is the amino acid sequence of position 18-27 of the HBV core antigen or variant sequences thereof, the amino acid sequence of position 141-151 of the HBV core antigen or variant sequences thereof, the amino acid sequence of position 117-125 of the HBV core antigen or variant sequences thereof, the amino acid sequence of position 88-94 of the HBV core antigen or variant sequences thereof, the amino acid sequence of position 88-96 of the HBV core antigen or variant sequences thereof, the amino acid sequence of position 183-191 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 201-210 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 204-212 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 370-379 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 251-259 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 260-269 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 335-343 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 338-347 of the HBV surface antigen or variant sequences thereof, the amino acid sequence of position 348-357 of the HBV surface antigen or variant sequences thereof, the

amino acid sequence of position 378-387 of the HBV surface antigen or variant sequences thereof; the amino acid sequence at position 10-17 of the Pre S1 antigen or variant sequences thereof, the amino acid sequence at position 109-123 of the Pre S2 antigen or variant sequences thereof, the amino acid sequence at position 152-161 or variant sequences thereof; the amino acid sequence at position 92-100 of the HBx antigen or variant sequences thereof, the amino acid sequence at position 99-108 of the HBx antigen or variant sequences thereof, the amino acid sequence at position 115-123 of the HBx antigen or variant sequences thereof, the amino acid sequence at position 133-141 of the HBx antigen or variant sequences thereof; the amino acid sequence at position 61-69 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 455-463 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 575-583 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 773-782 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 803-811 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 756-764 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 816-824 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 655-663 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 551-559 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 772-780 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 502-510 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 538-546 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 642-650 of the Pol antigen or variant sequences thereof, the amino acid sequence at position 646-654 of the Pol antigen or variant sequences thereof; and said amino acid sequence 3 is the amino acid sequence at position 14-24 of the B cell epitope derived from HBV Pre-S2 or variant sequences thereof, or the determinant a of HBS antigen.

3. An immunogen according to claim 1 or 2, characterized in that said amino acid sequence 1 is QYIKANSKFIGITE or variant sequences thereof, PADRE or variant sequences thereof; said amino acid sequence 2 is PLGFFPDH or variant sequences thereof,

MQWNSTALHQALQDP or variant sequences thereof, SILSKTGDPV or variant sequences thereof, VLQAGFFLL or variant sequences thereof, FLLTRILTI or variant sequences thereof, FLGGTPVCL or variant sequences thereof, LLCLIFLLV or variant sequences thereof, LLDYQGMLPV or variant sequences thereof, WLSLLVPFV or variant sequences thereof, GLSPTVWLSV or variant sequences thereof, KVLHKRTLGL or variant sequences thereof, VLHKRTLGL or variant sequences thereof, GLSAMSTTDL or variant sequences thereof, CLFKDWEEL or variant sequences thereof, VLGGRHKLK or variant sequences thereof, FLPSDFFPSV or variant sequences thereof, STLPETTVVRR or variant sequences thereof, EYLVSGVW or variant sequences thereof, GLYSSTVPV or variant sequences thereof, GLSRYVARL or variant sequences thereof, FLLSLGIHL or variant sequences thereof, ILRGTSFVYV or variant sequences thereof, SLYADSPSV or variant sequences thereof, KYTSFPWLL or variant sequences thereof, SLYADSPSV or variant sequences thereof, ALMPYACI or variant sequences thereof, YMDDVVLGA or variant sequences thereof, WILRGTSFV or variant sequences thereof, KLHLYSHPI or variant sequences thereof, FTQAGYPAL or variant sequences thereof, SLNFLGGTTV or variant sequences thereof, LLDYQGMLPV or variant sequences thereof, LLVPFVQWFV or variant sequences thereof, GLSPTVWLSV or variant sequences thereof, LLPIFFCLWV or variant sequences thereof, YVNTNMG or variant sequences thereof, YVNTNMGLK or variant sequences thereof, SILSKTGDPV or variant sequences thereof, GLSPTVWLSV or variant sequences thereof, SIVSPFIPLL or variant sequences thereof; and said amino acid sequence 3 is DPRVRGLYFPA or variant sequences thereof, or CTKPTDGNCT or variant sequences thereof.

4. An immunogen according to any one of claims 1 to 3, characterized in that said linking peptide consists of 3-7 amino acid residues.
5. An immunogen according to any one of claims 1 to 4, characterized in that the linking peptide is AAA, SSS or GGG.

6. An immunogen according to any one of claims 1 to 5, characterized in that the order for linking the amino acid sequence 1, the amino acid sequence 2 and the amino acid sequence 3 is amino acid sequence 1-amino acid sequence 2-amino acid sequence 3, amino acid sequence 1-amino acid sequence 3-amino acid sequence 2, amino acid sequence 2-amino acid sequence 1-amino acid sequence 3, amino acid sequence 2-amino acid sequence 3-amino acid sequence 1, amino acid sequence 3-amino acid sequence 1-amino acid sequence 2, or amino acid sequence 3-amino acid sequence 2-amino acid sequence 1.
7. An immunogen according to any one of claims 1 to 6, characterized in that said immunogen further comprises several modifying groups which can be alkylcarbonyl groups, or alkenylcarbonyl groups.
8. An immunogen according to any one of claims 1 to 7, characterized in that said immunogen further comprises two modifying groups.
9. An immunogen according to any one of claims 1 to 7, characterized in that said immunogen further comprises one modifying group.
10. An immunogen according to any one of claims 7 to 9, characterized in that said alkylcarbonyl is one to five alkylcarbonyl groups selected from a group consisting of  $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$ ,  $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$  and  $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$ ; and the said alkenylcarbonyl is one or five alkenylcarbonyl groups selected from a group consisting of  $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}-$ ,  $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}-(\text{CH}_2)_7\text{CO}-$  and  $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}-$ .
11. An immunogen according to any one of claims 7 to 10, characterized in that said modifying group is covalently linked to any amino acid residue of said polypeptide sequence.

12. An immunogen according to any one of claims 7 to 11, characterized in that said modifying group is covalently linked to N-terminal  $\alpha$ -amino group, C-terminal  $\alpha$ -carboxyl group or any side chain group of an amino acid residue of said polypeptide sequence.
13. An immunogen according to claim 12, characterized in that said modifying group is linked the N-terminal  $\alpha$ -amino group of said polypeptide sequence via a linking peptide KSS, wherein the N-terminal  $\alpha$ -amino group is linked to the C-terminus of the linking peptide KSS via a peptide bond, and said modifying group is covalently linked to the  $\epsilon$ -amino group on the linking peptide KSS.
14. An immunogen according to claim 12 or 13, characterized in that said modifying group is covalently linked to an amino group, carboxyl group or hydroxyl group on said side chain group.
15. An immunogen according to claim 12, characterized in that said modifying group is covalently linked to the  $\epsilon$ -amino group on the N-terminal lysine.
16. An immunogen according to claim 13, characterized in that the  $\alpha$ -amino group of said linking peptide KSS is further covalently linked to one of said modifying groups.
17. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{10}\text{COKSSPADREGGSLNFLGGTTVSSSD PRVRGLYFPA}$ .
18. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAAALLCLIFLLV GGGDPRVRGLYFPA}$ .
19. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSPADREAAALLDYQGMLPVGGG}$

DPRVRGLYFPA.

20. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)\text{-CO}$ ,  $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}_7\text{KSSQYIK ANSKFIGITEGGG}$ .
21. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}$  FLPSDFFPSVAAADPRVRGLYFPA.
22. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}$  KSSPADREGGGWLSLLVPFVSSSDPRVRGLYFPA.
23. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAAA}$  FLPSDFFPSVGGGDPRVRGLYFPA.
24. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSPADREAAAFPSD FFPSVGGGDPRVRGLYFPA}$ .
25. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSPADREGGGLLPFVQ WFFVSSSDPRVRGLYFPA}$ .
26. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSPADREAAAGLSPTVW}$  LSVGGGDPRVRGLYFPA.
27. An immunogen according to any one of claims 1 to 16, characterized in that the primary

structure thereof is  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSPADREAAALLPIFF CLWVG GGDPRVRGLYFPA}$ .

28. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure is thereof  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSQYIKANSKFI GITEAAAYVNTNMGG GGDPRVRGLYFPA}$ .

29. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSQYIKANSKFIGITEAAA FLPSDFFPSVGGGDPRVRGLYFPA}$ .

30. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEGGG FLPSDFFPSVSSSDPRVRGLYFPA}$ .

31. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAAA YVNTNMGLKGGGDPRVRGLYFPA}$ .

32. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAAA PLGFFPDHGGG DPRVRGLYFPA}$ .

33. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAAA MQWNSTALHQALQDPGGGDPRVRGLYFPA}$ .

34. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSPDAREAAASILSKTGD PVGGGDPRVRGLYFPA}$ .

35. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSPADREAAVLQAGFFLLGGG DPRVRGLYFPA}$ .
36. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSPADRESSSFLLTRILTIGGG DPRVRGLYFPA}$ .
37. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSPADREAAFLGGTPVCLGGG DPRVRGLYFPA}$ .
38. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAAAGLSPTVW LSVGGGDPRVRGLYFPA}$ .
39. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAAASIVS PFIPLLGGGDPRVRGLYFPA}$ .
40. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3(\text{CH}_2)_{16}\text{COKSSPADREAAASTLPETTVVRR GGGDPRVRGLYFPA}$ .
41. An immunogen according to any one of claims 1 to 16, characterized in that the primary structure thereof is  $\text{CH}_3-(\text{CH}_2)_{14}\text{COKSSQYIKANSKFIGITEAA AFLPSD FFPSVGGGCTKPTDGNCT}$ .
42. A method for designing, screening and synthesizing an immunogen according to any one of claims 1 to 41, comprising epitope-based vaccine design (EBVD), molecular simulation, molecular design, screening system and solid-phase synthesis of polypeptide, wherein in the polypeptide solidphase synthesis, the molar ratio of resin to each amino acid or palmitic



acid feed is from 1:2 to 1:8, the double coupling is used for linking arginine, asparagine and palmitic acid component, and the reaction temperature is from 20 to 40 °C.

43. A method according to claim 42, wherein the molar ratio of resin to each amino acid or palmitic acid feed is 1:4, and the reaction temperature is 30 °C.
44. A method for preparing an immunogen according to any one of claims 1-41, characterized in that said method comprises the following steps: (1) synthesizing an immunogen-resin by polypeptide solid synthesis, wherein said immunogen-resin represents the immunogen bound to resin; (2) cleaving said immunogen-resin to obtain a cleavage solution; (3) preliminarily purifying the cleavage solution of the step (2) by size exclusion chromatography; and (4) purifying by reversed phase chromatography to obtain the immunogen.
45. A method according to claim 44, characterized in that TFA cleaving solution is used in the step (2), and the cleavage conditions are that the concentration of the immunogen-resin is lower than 100 mg/ml, the reaction temperature is from 15 to 50 °C, and the reaction time is from 0.5 to 3 hours.
46. A method according to any one of claims 44 to 45, characterized in that said TFA cleaving solution is composed of 0.75 g phenol, 0.25 ml dithioglycol, 0.5 ml phenyl methyl thioether, 0.5 ml deionized water, and 10.0 ml TFA; and said cleavage conditions are that the concentration of the immunogen-resin is 40 mg/ml, the reaction temperature is 25 °C, and the reaction time is 1.5 hours.
47. A method according to claim 44, characterized in that in the size exclusion chromatography in the step (3), Sephadex LH20 is used as the column packing, and dimethyl sulphoxide is used as the mobile phase.

48. A method according to claim 44, characterized in that in the reversed phase chromatography in the step (4), POROS 50 R1, POROS 50R2, SOURCE 30 RPC or Dleta Pak C18 is used as column packing.
49. A method according to claim 44 or 47, characterized in that gradient eluting is employed in the reversed phase chromatography in the step (4), wherein the mobile phase is an aqueous solution of acetonitrile/TFA, acetonitrile/HCl, ethanol/TFA, ethanol/HCl or ethanol/phosphoric acid.
50. A method according to claim 44 or 47 or 48, characterized in that the column temperature of said reversed phase chromatography is from 20 to 60 °C.
51. A method according claim 50, characterized in that the column temperature of said reversed phase chromatography is from 28 to 40 °C.
52. A method according claim 51, characterized in that the column temperature of said reversed phase chromatography is from 32 to 36 °C.
53. A method according claim 52, characterized in that the column temperature of said reversed phase chromatography is 34 °C.
54. Use of an immunogen according to any one of claims 1 to 41 in the manufacture of a vaccine or a medicament for treatment of the chronic HBV persistent infection state and the relevant secondary diseases such as liver cirrhosis, liver cancer, etc.
55. Use according to claim 54, characterized in that said chronic HBV persistent infection state occurs in a patient with chronic hepatitis B or a carrier of hepatitis B virus.
56. A vaccine for treatment of hepatitis B, characterized in that said vaccine comprises an

immunogen according to any one of claims 1 to 41.

57. A vaccine for treatment of hepatitis B, characterized in that said vaccine comprises an immunogen according to any one of claims 1 to 41 and pharmaceutically acceptable auxiliary materials, adjuvants and/or carriers.
58. A vaccine for treatment of hepatitis B according to claim 54 or 55, characterized in that said vaccine is in any pharmaceutically acceptable formulation.
59. A vaccine for treatment of hepatitis B according to claim 54, 55, 56 or 57, characterized in that the formulation of said vaccine is injection formulation, percutaneous formulation, oral formulation, inhalant formulation or suppository formulation.
60. A vaccine for treatment of hepatitis B according to claim 58, characterized in that said vaccine is in liquid dosage form, suspension dosage form, liquid liposome dosage form or lyophilized liposome dosage form.
61. A vaccine for treatment of hepatitis B according to claim 60, characterized in that said liquid dosage form is an ethanol solution dosage form.
62. A vaccine for treatment of hepatitis B according to claim 60, characterized in that said liquid liposome dosage form or lyophilized liposome dosage form comprises phospholipids.
63. A vaccine for treatment of hepatitis B according to claim 62, characterized in that said liquid liposome dosage form or lyophilized liposome dosage form further comprises cholesterol.
64. A vaccine for treatment of hepatitis B according to claim 62 or 63, characterized in that said liquid liposome dosage form or lyophilized liposome dosage form further comprises

vitamin E.

65. A vaccine for treatment of hepatitis B according to claims 62 to 64, characterized in that said liquid liposome dosage form or lyophilized liposome dosage form further comprises palmitic acid.
66. A vaccine for treatment of hepatitis B according to claim 60 or 65 or 65, characterized in that the molar ratio of immunogen : phospholipids : cholesterol : vitamin E : palmitic acid in the vaccine is 0.1-0.5 : 40-80 : 0-40 : 0-10 : 0-10.
67. A vaccine for treatment of hepatitis B according to claim 66, characterized in that the molar ratio of immunogen : phospholipids : cholesterol : vitamin E : palmitic acid in the vaccine is 0.2-0.4 : 60 : 20 : 6 : 6.
68. A vaccine for treatment of hepatitis B according to claim 67, characterized in that the molar ratio of immunogen : phospholipids : cholesterol : vitamin E : palmitic acid in the vaccine is 0.3-0.36 : 60 : 20 : 6 : 6.
69. A vaccine for treatment of hepatitis B according to any one of claim 60 or 62, characterized in that said phospholipids are soybean phospholipids or lecithin.
70. A method for preparing a vaccine for treatment of hepatitis B according to any one of claim 60 or 62, characterized in that said method comprises using double emulsifying method to prepare the liposome.
71. A vaccine for treatment of hepatitis B according to any one of claim 60 or 62, characterized in that said lyophilized liposome dosage form further comprises human albumin, mannitol and phosphates.

72. A vaccine for treatment of hepatitis B according to claim 60 or 62 to 71, characterized in that said lyophilized liposome dosage form comprises an immunogen according to claims 1 to 41, phospholipids, cholesterol, palmitic acid, vitamin E, mannitol, human albumin,  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{PO}_4$  in a molar ratio of 0.01-0.1 : 5-15 : 1-7 : 0.5-1.5 : 0.5-1.5 : 70-150 : 0.1-0.3 : 1-10 : 1-10.